

AMENDMENTS TO THE SPECIFICATION

Kindly amend the title of the application as follows.

PROCESS METHOD FOR PRODUCING ~~VIRUS VECTOR~~ VIRAL VECTORS

Kindly insert the following heading and paragraph at page 1, line 4 of the English language specification.

Cross-Reference to Related Applications

This application is the U.S. National Stage of International Application No. PCT/JP2005/000708, filed January 20, 2005, which, in turn, claims the benefit of Japanese Patent Application No. 2004-014654, filed January 22, 2004.

Kindly amend the paragraph starting at page 7, line 23 of the English language specification as follows.

Fig. 5 shows the procedure for constructing ~~pCALNdLWE-zeo-NP(Z)~~ pCAGGS-NP.

Kindly amend the paragraph starting at page 12, line 12 of the English language specification as follows.

Virus-producing cells preferably express the second exogenous protease at a high level. For example, virus-producing cells can exogenously express the second protease that cleaves a modified viral protein. When the second protease is expressed at a high

level, the cleavage of the modified viral protein can be enhanced to further improve the efficiency of virus production. To achieve this, an expression vector encoding the second protease is introduced into cells. A desired promoter for expressing genes in mammalian cells can be used to express the second protease. In addition, such an expression vector may be constructed using a recombinase target sequence or inducible promoter so that the expression of the second protease is induced depending on a stimulation (described below).

Kindly amend the paragraph starting at page 17, line 27 of the English language specification as follows.

The chicken β -actin promoter includes a DNA fragment with promoter activity that comprises a transcription initiation site for the genomic DNA of the chicken β -actin gene and a TATA box (Ann. Rev. Biochem. 50, 349-383, 1981) and CCAAT box (Nucl. Acids Res. 8, 127-142, 1980). The nucleotide sequence of the chicken β -actin gene promoter has been reported by, for example, T. A. Kost et al. (Nucl. Acids Res. 11, 8287-8286 8301, 1983). In the chicken β -actin promoter, the region from G (guanine) at position -909 to G (guanine) at position -7 upstream of the translation initiation codon (ATG) of the original β -actin structural gene is considered as an intron. Since this intron has transcription-promoting activity, it is preferable to use a genomic DNA fragment comprising at least a portion of this intron. Specifically, examples of such chicken β -

actin promoters include, for example, DNA comprising the nucleotide sequence of SEQ ID NO: 18. For the intron acceptor sequence, an intron acceptor sequence from a different gene can be used. For example, a splicing acceptor sequence of rabbit β -globin may be used. More specifically, the acceptor site of the second intron, which is located immediately before the initiation codon of rabbit β -globin, can be used. Specifically, such acceptor sequences include, for example, DNA comprising the nucleotide sequence of SEQ ID NO: 19. A CA promoter of the present invention is preferably a DNA in which a chicken β -actin promoter comprising a portion of the intron is linked downstream of a CMV IE enhancer sequence and a desired intron acceptor sequence is added downstream thereof. An example is shown in SEQ ID NO: 20. To express a protein, the last ATG in this sequence is used as the start codon and the coding sequence for the modified viral protein may be linked thereto.

Kindly amend the paragraph starting at page 18, line 28 of the English language specification as follows.

Variants of the CMV IE enhancer sequence and chicken β -actin promoter as described above include sequences that have equivalent promoter activity, and which comprise a nucleotide sequence having a substitution, deletion, and/or insertion of 30% or less, preferably 20% or less, more preferably 15% or less, more preferably 10% or less, more preferably 5% or less, more preferably 3% or less of the nucleotides in the CMV IE

enhancer sequence of SEQ ID NO: 17 and the chicken β -actin promoter of SEQ ID NO: 18. These sequences exhibit high homology to the nucleotide sequence of either SEQ ID NO: 17 or 18. High homology nucleotide sequences include those with an identity of, for example, 70% or higher, more preferably 75% or higher, even more preferably 80% or higher, still more preferably 85% or higher, yet more preferably 90% or higher, even still more preferably 93% or higher, yet still more preferably 95% or higher, yet still even more preferably 96% or higher. The nucleotide sequence identity can be determined, for example, using the BLAST program (Altschul, S. F. et al., 1990, J. Mol. Biol. 215: 403-410). For example, search is carried out on the BLAST web page of NCBI (National Center for Biotechnology Information) using default parameters, with all the filters including Low Complexity turned off (Altschul, S. F. et al. (1993) Nature Genet. 3:266-272; Madden, T. L. et al. (1996) Meth. Enzymol. 266:131-141; Altschul, S. F. et al. (1997) Nucleic Acids Res. 25:3389-3402; Zhang, J. & Madden, T. L. (1997) Genome Res. 7:649-656). Sequence identity can be determined, for example, by comparing two sequences using the blast2sequences program to prepare an alignment of the two sequences (Tatiana A et al. (1999) FEMS Microbiol Lett. 174:247-250). Gaps are treated in the same way as mismatches. For example, an identity score is calculated in view of the entire nucleotide sequences of SEQ ID NOs: 17 and 18. Specifically, the ratio of the number of identical nucleotides in the alignment to the total number of nucleotides of SEQ ID NO: 17 or 18 is calculated. Gaps outside of SEQ ID NO: ~~1 or 2~~ 17 or 18 in the

alignment is excluded from the calculation.

Kindly amend the paragraph starting at page 20, line 36 of the English language specification as follows.

To construct a vector whose expression is induced in a recombination enzyme-dependent manner, a DNA fragment flanked by a pair of target sequences of the recombination enzyme is inserted between a promoter and the coding sequence of a modified viral protein. In this state, due to interference by the inserted DNA fragment, the modified viral protein is not expressed. However, when the recombination enzyme acts on the DNA, the target sequence-flanked DNA is excised, which enables the ~~recombination enzyme~~ modified viral protein to be expressed from the promoter. As described above, expression from the promoter can be induced by a recombination enzyme. The DNA flanked by the recombination enzyme target sequences is preferably designed to contain a transcription termination signal and/or stop codon so that the expression of the downstream gene is definitely inhibited in the absence of the recombination enzyme action. An appropriate marker gene can also be inserted into the DNA flanked by the target sequences of the recombination enzyme.

Kindly amend the paragraph starting at page 23, line 5 of the English language specification as follows.

The minus-strand RNA virus used in the present invention particularly includes single-stranded minus-strand RNA viruses (also referred to as non-segmented minus-strand RNA viruses), which have a single-stranded negative strand [*i.e.*, a minus strand] RNA as the genome. Such viruses include viruses belonging to Paramyxoviridae (including the genera *Paramyxovirus*, *Morbillivirus*, and *Rubulavirus*), Rhabdoviridae (including the genera *Vesiculovirus*, *Lyssavirus*, and *Ephemerovirus*), Filoviridae, ~~Orthomyxoviridae, (including Influenza viruses A, B, and C, and Thogoto-like viruses),~~ Bunyaviridae ~~(including the genera *Bunyavirus*, *Hantavirus*, *Nairovirus*, and *Phlebovirus*),~~ Arenaviridae, and the like.

Kindly amend the paragraph starting at page 32, line 36 of the English language specification as follows.

In a method of transcribing the minus-strand RNA virus genome by a bacteriophage RNA polymerase, it is possible to use 0.1 to 2 μg (more preferably 0.5 μg) of an NP-expressing plasmid, 0.1 to 2 μg (more preferably 0.5 μg) of a P-expressing plasmid, 0.5 to 4.5 μg (more preferably 2.0 μg) of an L-expressing plasmid, 0.1 to 5 μg (more preferably 0.5 μg) of a modified-F-expressing plasmid, a T7 RNA polymerase-expressing plasmid (for example, 0.5 μg), and 0.5 to 5 μg (more preferably 5 μg) of a viral genomic RNA-encoding plasmid (plus or minus strand). To produce SeV, for example, the plasmids described in the Examples can be used in the following amounts:

| | |
|---|---|
| pCAGGS-NP | 0.1 to 2 µg (more preferably, 0.5 µg) |
| pCAGGS-P | 0.1 to 2 µg (more preferably, 0.5 µg) |
| pCAGGS-L(TDK) | 0.5 to 4.5 µg (more preferably, 2.0 µg) |
| pCAGGS-F5R | 0.1 to 5 µg (more preferably, 0.5 µg) |
| <u>pCAGGS-T7</u> | <u>for example, 0.5 µg</u> |
| pCAGGS-SeV <u>pSeV(TDK)18+GFP</u> | 0.5 to 5 µg (more preferably, 5 µg) |
| (pCAGGS-SeV/ΔF-GFP) <u>(pSeV/ΔF-GFP)</u> | |

Kindly insert the sequence listing enclosed herewith at the end of the specification.